

Regulation of HIF1 α under Hypoxia by APE1/Ref-1 Impacts CA9 Expression: Dual-Targeting in Patient-Derived 3D Pancreatic Cancer Models

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Abstract

Half of all patients diagnosed with pancreatic ductal adenocarcinoma (PDAC) die within a year despite extensive surgery and/or a highly aggressive chemotherapy regimen. Several mechanisms are proposed to play a role in PDAC therapeutic resistance, including reactive stroma and hypoxia. Hypoxia signaling creates a more aggressive phenotype with increased metastatic potential and impaired therapeutic efficacy. Carbonic anhydrase IX (CA9) functions as part of the cellular hypoxia response to regulate intracellular pH, promoting cell survival. Apurinic/Apyrimidinic Endonuclease-1-Reduction/oxidation Effector Factor 1 (APE1/Ref-1) is a multi-functional protein with two major activities: an endonuclease function in DNA base excision repair and a redox signaling function that reduces oxidized transcription factors, enabling them to bind to their DNA target sequences. APE1/Ref-1 regulates several transcription factors involved in survival mechanisms, tumor growth, and hypoxia signaling. We explored the mechanisms underlying PDAC cell responses to hypoxia and modulation of APE1/Ref-1 redox signaling control of hypoxia inducible factor 1 alpha (HIF1 α), a critical factor in hypoxia-induced CA9 transcription. **We hypothesized that obstructing the HIF-CA9 axis at two points via APE1/Ref-1 inhibition and CA9 inhibition will result in enhanced PDAC cell killing under hypoxic conditions.**

Methods: We performed qRT-PCR and Western Blots to confirm changes in CA9 expression in PDAC cells following APE1/Ref-1 inhibition and hypoxia exposure. Proliferation assays were used to assess cell killing following inhibition of APE1/Ref-1 and CA9 under hypoxia. Ex vivo 3-Dimensional co-culture models including both tumor and CAFs were used to examine whether we could enhance the efficacy of APE1/Ref-1 and/or CA9 inhibition with a dual-targeting approach to kill tumor spheroids.

Results: HIF1 α -mediated induction of CA9 is significantly diminished in PDAC cells following APE1/Ref-1 redox inhibition. Additionally, dual-targeting of APE1/Ref-1 and CA9 reduces PDAC tumor cell growth under hypoxic conditions and in 3D tumor co-cultures.